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Circadian Rhythm of Serum Erythropoietin in Multiple Myeloma

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The diurnal rhythm in the circulating serum levels of erythropoietin (EPO) were determined in a group of 20 adult clinically-healthy subjects, in a group of 10 patients with myeloma without renal impairment and 10 patients with myeloma and renal failure. Venous blood samples were drawn during the span of a whole day and every 4 hr, starting from midnight, for the measurement of serum EPO levels by radioimmunoassay (RIA). Statistical analysis was carried out by means of the "cosinor" method. Results show that the controls and the myeloma patients without renal insufficiency present significant ($P < 0.05$) circadian rhythms in serum EPO levels; no rhythm ($P < 0.05$) was detected in patients with myeloma and renal failure. Patients with myeloma and renal failure have significant ($P < 0.05$) lower mean daily levels and diurnal fluctuations of EPO than the other groups, whereas the patients with myeloma without renal involvement present higher ($P < 0.05$) mean daily levels and lower ($P < 0.05$) diurnal variations of EPO than controls; no differences ($P > 0.05$) exist between the groups regarding peaks of rhythms. These data confirm the existence of a physiological circadian rhythm in serum EPO concentrations, with maximum in the afternoon, and they suggest that renal failure is an important cause of anemia and loss of EPO circadian rhythm in patients with myeloma.

Key words: circadian rhythm, erythropoietin, multiple myeloma, renal failure

INTRODUCTION

Erythropoietin (EPO), a glycoprotein produced mainly by the peritubular cells of the kidney and also by the liver, has a predominant effect on the committed erythroid cells, colony-forming unit-erythroid, promoting their proliferation and differentation into proerythroblasts; it may also stimulate the differentation of a more primitive erythroid progenitor, the burst-forming unit-erythroid, in association with so-called burst-promoting activity; EPO also interacts with other hematopoietic growth factors to promote the production of megakaryocytes [1]. EPO is detectable in the serum [1,2] and shows large fluctuations during the 24-hr period, with a well-marked circadian rhythm [2,3] with maximum levels in the afternoon. Anemia is a common complication of multiple myeloma (MM), and it is suggested that a deficit in synthesis and activity of EPO could be responsible, especially in patients with renal failure [4]. The aim of this study was to investigate diurnal variations of serum-circulating EPO levels in patients with MM, with and without renal insufficiency, in order to verify whether these two diseases are associated with quantitative or qualitative changes in circadian rhythm.

MATERIALS AND METHODS

Three groups of subjects were considered for the study: group A, 20 clinically healthy normocytemic subjects, 13 males and 7 females, with mean age of 65.3 ± 6.4 years, Hb of 13.5 ± 1.7 g/dl, and serum creatinine of 1.23 ± 0.14 mg/dl; group B, 10 patients with MM without renal impairment, 7 males and 3 females, with mean age of 66.5 ± 4.9 years, mean Hb of 12.8 ± 1.3 g/dl, and mean serum creatinine of 1.58 ± 0.34 mg/dl; and group

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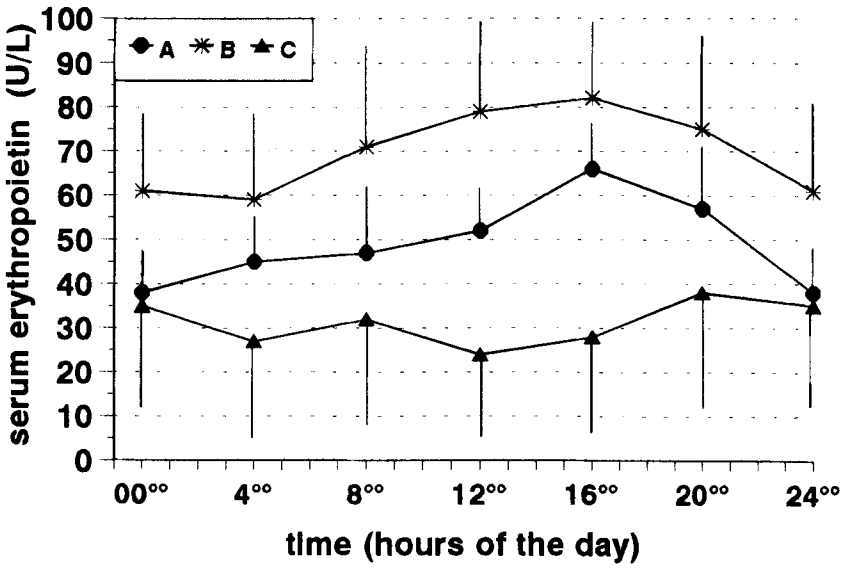

groups	p	mesor (SE)	amplitude (95% CL)	acrophase (95% CL)
A) controls	<0.01	50.2 (4.1)	14.2 (9.6-18.6)	6:08pm (4:18pm-7:58pm)
B) MM without renal failure	<0.05	70.7 (6.7)	10.3 (3.1-17.4)	6:44pm (4:06pm-9:14pm)
C) MM with renal failure	>0.05	32.5 (8.3)	3.2 (- ; -)	9:32pm (- ; -)

Fig. 1. Diurnal fluctuations of serum erythropoietin levels in the three studied groups, and results of statistical analysis by means of the "mean-group cosinor" method.

C, 10 patients with MM and renal failure, 6 males and 4 females, with mean age of 69.3 ± 5.9 years, Hb of 10.2 ± 1.5 g/dl, and serum creatinine of 3.45 ± 0.68 mg/dl. All patients met the diagnostic criteria for MM of the Southwest Oncology Group, and no subjects had received drugs or blood transfusions in the last 2 months. The study was performed after 3 days of synchronization period in hospital, with sleep and/or rest period from 10:00 PM–6:00 AM, and meals at 8:00 AM, 12:30 PM, and 6:30 PM with free diet. To study the circadian rhythm of EPO, venous blood samples were drawn from a peripheral vein of each subject during the span of a whole day and every 4 hr, starting at midnight. Serum levels of EPO were assayed in each sample by RIA [5]; normal range using this technique is 5–100 U/l.

The time-related values of EPO were subjected to statistical analysis using chronograms (mean ± 1 SD), and to inferential circadian statistical analysis by means of the "mean-group cosinor" method [6], which is able to detect a significant ($P < 0.05$) circadian rhythm and the rhythm parameters: mesor (average level of rhythm), amplitude (length from mesor to acrophase), and acrophase (peak of rhythm). Circadian rhythms of EPO were compared among groups by Hotelling's statistic test [6].

RESULTS

Serum levels of EPO fluctuated during the day in each group. When the data were analyzed by "cosinor" method, the controls (group A) and MM patients without renal failure (group B) presented significant ($P < 0.05$) circadian rhythms for EPO, with the highest values in the late afternoon and the lowest values at night; no rhythm ($P > 0.05$) was detected in MM patients with renal failure (group C). Patients in group C had significantly ($P < 0.05$) lower mesor and amplitude of EPO than the other groups; patients in group B had significantly ($P < 0.05$) higher mesor and lower amplitude of EPO than controls. No significant ($P > 0.05$) differences were found regarding the acrophases of the three groups. Figure 1 illustrates the results in detail.

DISCUSSION

Several variables have been investigated from a chronobiological circadian point of view in MM [7,8], and this study is a further contribution in this field.

The present observations confirm that serum EPO presents in healthy subjects a definite circadian rhythm, with

its peak in the afternoon [2,3]. The mechanisms behind the observed circadian rhythm are still unclear. It is hypothesized that, since several of the hormones secreted or regulated by the pituitary gland show circadian rhythm, their rhythms may be involved in the control of EPO circadian rhythm; moreover, changes in release rate and in metabolism of EPO and variations of blood flow through the kidney during the day have to be considered [2,5].

The diurnal rhythm of EPO is preserved in patients with MM without renal impairment, suggesting, together with the observation of a higher mesor, that EPO does not seem to have a fundamental role in the pathogenesis of anemia in MM without renal impairment [4]. On the contrary, only in patients with renal failure has a defect in production and activity of EPO been suggested as the cause of anemia in MM [4,9]. In effect, renal failure is characterized by a real insufficiency in EPO production [9,10], such as confirmed by the reduced mesor in our patients. Moreover, renal failure, and not MM, seems to be responsible for the loss in EPO circadian rhythm. The reduced amplitude, with constant daily serum levels, represents, from a chronobiological point of view, a compensatory mechanism: constant levels of any biological active substance have the same effectiveness at higher, but fluctuating, levels [7].

Finally, the demonstrations that EPO presents its higher values in the afternoon and that this rhythm is lost in MM patients with renal failure suggest that therapy with recombinant human EPO should be administered at this time of the day, according to the physiological circadian rhythm: in effect, the evening intravenous administration of recombinant human EPO in patients with renal failure seems to insure a quicker therapeutical success [10].

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REFERENCES

1. Groopman JE, Molinas JM, Scadden DT: Hematopoietic growth factors. N Engl J Med 321:1449–1459, 1989.
2. Wide L, Bengtsson C, Birgegård G: Circadian rhythm of erythropoietin in human serum. Br J Haematol 72:85–90, 1989.
3. Allegra A, Buemi M, Corica F, Giacobbe MS, Frisina N, Ceruso D: Erythropoietin: Present and future. Recenti Prog Med 90:531–536, 1989.
4. Takagi M, Miyamoto Y, Kosaka M, Saito S: Clinical significance of serum erythropoietin in patients with multiple myeloma. Rinsho Ketsueki 33:1151–1157, 1992.
5. Cotes PM: Immunoreactive erythropoietin in serum. Br J Haematol 50:427–438, 1982.
6. Nelson W, Tong YL, Lee JK, Halberg F: Method for cosinorhythmometry. Chronobiologia 6:303–323, 1979.
7. Pasqualetti P, Casale R, Colantonio D, Collacciani A, Natali G: Diurnal variation of serum β_2-microglobulin in multiple myeloma. Life Sci 49:435–438, 1991.
8. Pasqualetti P, Collacciani A, Casale R: Chronobiological circadian aspects of serum lactate dehydrogenase and serum thymidine kinase in monitoring multiple myeloma. Am J Hematol 51:176–177, 1996.
9. Ting WC, Cavill J, Jacobs A: Anaemia in patients with myelomatosis. Br J Cancer 45:887–894, 1982.
10. Buemi L, Allegra A, Laganà A, Aloisi C, Privitera M, Morabito N, Frisina N: Effects of the evening IV administration of erythropoietin in haemodialyzed patients. Eur Rev Med Pharmacol Sci 15:195–197, 1993.